

In Vivo Evaluation of *Saccharomyces cerevisiae* for Reducing Milk Aflatoxin M1 in Dairy Cattle

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ABSTRACT

Aflatoxin B₁ (AFB₁) contamination in livestock feed poses a significant risk to animal health and dairy product safety due to its conversion to aflatoxin M₁ (AFM₁) in milk. This study evaluated the efficacy of *Saccharomyces cerevisiae* in mitigating aflatoxin toxicity and reducing AFM₁ carry-over in milk of lactating Sahiwal cows. Twenty cows, uniform in body weight (400-500 kg), lactation number (3-6), and early lactation stage (1-4 months), were randomly assigned to four treatment groups. Animals in T₁ group received basal feed (TMR), while T₂-T₄ groups received *Saccharomyces cerevisiae* in feed @ 0.05, 0.10 and 0.20%, respectively. Milk yield, AFM₁ excretion, carry-over rate, somatic cell count (SCC), biochemical, and haematological parameters were measured. Yeast supplementation significantly improved dry matter intake and milk yield, reduced AFM₁ excretion and carry-over rate, and decreased SCC (p<0.05). Biochemical and haematological parameters indicated alleviation of aflatoxin-induced hepatic damage and suppression of red and white blood cells. The highest efficacy was observed at 0.1-0.2% yeast inclusion. These findings demonstrate that *Saccharomyces cerevisiae* effectively reduces AFM₁ contamination in milk while improving animal performance and health, offering a practical strategy for enhancing milk safety in aflatoxin-exposed dairy systems.

Key words: Aflatoxin B₁, Aflatoxin M₁, Dairy cattle, Milk safety, Mycotoxin detoxification *Saccharomyces cerevisiae*.

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INTRODUCTION

Aflatoxins are toxic secondary metabolites produced by *Aspergillus* species, predominantly *Aspergillus flavus* and *Aspergillus parasiticus*, commonly contaminating livestock feeds under warm and humid conditions. Among them, aflatoxin B₁ (AFB₁) is the most potent, classified as a Group I carcinogen, and poses serious threats to animal health, productivity, and food safety. In dairy cattle, ingestion of AFB₁ leads to hepatic damage, suppressed immunity, reduced feed intake, decreased milk production, and conversion of AFB₁ into aflatoxin M₁, a carcinogen metabolite excreted into milk. AFM₁ contamination in milk poses a significant public health concern, necessitating strategies to mitigate its transfer from feed to milk (Summa *et al.*, 2025).

Several approaches have been explored to reduce aflatoxin toxicity, including physical, chemical, and biological methods. Among biological strategies, yeast-supplementation, particularly *Saccharomyces cerevisiae*, has shown promise due to its ability to bind aflatoxins in the gastrointestinal tract, modulate gut microbiota, improve nutrient utilization, enhance gut morphology, and reduce inflammatory responses (Brugger *et al.*, 2023). However, the efficacy of yeast varies among strains and products; optimal inclusion levels for reducing AFM₁ in milk require systemic evaluation. This study was aimed to assess the efficacy of *Saccharomyces cerevisiae* supplementation in mitigating aflatoxin toxicity, reducing AFM₁ carry-over into milk, and improving performance, milk quality, and health parameters in lactating Sahiwal cows.

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MATERIALS AND METHODS

Experimental Design

A biological trial was conducted to evaluate the efficacy of the most effective aflatoxin detoxifying agent identified during the *in vitro* study. A total of 20 lactating Sahiwal cows of nearly uniform body weight (400-500 kg), lactation number (3-6), and in the early lactation stage (1-4 months) were selected for the experiment. The animals were randomly allotted into

four treatment groups (T₁-T₄), with five animals per treatment, following a completely randomized design. Each animal constituted one experimental unit. Animals in T₁ group received basal feed (TMR), while those in T₂, T₃ and T₄ groups were fed basal diet supplemented with *Saccharomyces cerevisiae* (Kothari Fermentation and Biochemical Limited, Bulandshahar, UP, India) @ 0.05, 0.10 and 0.20%, respectively.

A TMR containing roughage and concentrate in a 60:40 ratio was formulated. The roughage component consisted of berseem, oats, and wheat straw, while the concentrate mixture comprised maize grain, barley grain, oilseed cakes, wheat bran, de-oiled rice bran, mineral mixture, and common salt. Animals were housed individually throughout the experiment period and had *ad libitum* access to clean water. The average daily milk yield of animals in all treatment groups was recorded during the experimental period.

Sample Collection and Carry-over of Aflatoxin M₁ into Milk

Milk yield of individual animals was recorded daily during morning (4:00 AM) and evening (4:00 PM) milking. Milk samples were collected on days 2, 4, 6, 8, and 10 of the collection period. For each animal, 100 mL milk from both morning and evening milking was pooled to prepare a representative daily sample. The milk samples were stored at -20°C until further analysis. Milk yield was recorded for 10 consecutive days; morning and evening yields were summed to calculate total daily milk production per animal.

The carry-over of aflatoxin M₁ (AFM₁) from feed to milk was calculated using the following formula: Carry-over (%) = (Total AFB₁ intake through feed / Total AFM₁ in milk) × 100

Preparation and Analysis of Aflatoxin-Contaminated Feed

The aflatoxin B₁ (AFB₁) content in feed samples was estimated using Thin Layer Chromatography (TLC) following the method described by Pons *et al.* (1966). Quantification of aflatoxin was carried out using UV-spectrophotometry. The concentration of aflatoxin M₁ (AFM₁) in milk samples was determined using an aflatoxin toxinometer (VIACAM, USA) as per the manufacturer's instructions.

Blood Collection for Haemato-Biochemical Parameters

On the final day of the experiment, blood samples were collected aseptically from each animal via jugular venipuncture. The collected blood was divided into two portions: one for haematological analysis with anticoagulant and the other for biochemical analysis without anticoagulant. Serum was separated from later samples by centrifugation and stored at -20°C until analysis.

Haematological parameters, *viz.*, RBC count (using an improved Neubauer's counting chamber), total leukocyte count (WBC, using Turk's fluid) and packed cell volume (PCV, by microhematocrit method) were determined as per Schalm

et al. (1975). Erythrocyte indices, MCV, MCH, MCHC were calculated using standard formulae.

The biochemical parameters, *viz.*, serum total protein (Biuret method), glucose (enzymatic method), blood urea nitrogen (BUN, urease enzymatic method), and total cholesterol (Wybenga *et al.*, 1970 method) were estimated using assay kits of Span Diagnostics, Ltd., Sachin, Surat (India), while creatinine (modified Jaffe's kinetic method) was assayed using kit of Tulip Diagnostics Ltd., India) on a P-2011 Semi-Automatic Biochemistry Analyser. Further, serum Acetylcholinesterase (AChE, Ellman *et al.* 1961 method), Glutamic pyruvic transaminase (SGPT) and Glutamic oxaloacetic transaminase (SGOT, Reitman and Frankel, 1957 method), Alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) activities were also estimated using a commercial kits (Span Diagnostics Ltd., Surat, India) on a P-2011 Semi-Automatic Biochemistry Analyser.

Somatic Cell Count (SCC) in Milk

Somatic cell count was determined by the direct microscopic method. Fresh milk (0.01 mL) was spread uniformly over a 10 mm × 10 mm marked glass slide and dried at 30-40 °C. Fat globules were removed by dipping slides in xylene, followed by staining with methylene blue for 15 min. SCC was counted under a microscope (15x40X) in 50 microscopic fields and expressed as cells per millilitre using an appropriate microscopic factor.

Statistical Analysis

Data was analysed using one-way analysis of variance (ANOVA) in SPSS software (version 20.0). Treatment means were compared using Duncan's multiple range test. Differences were considered significant at p<0.05.

RESULTS AND DISCUSSION

Yeast has been reported to improve animal performance by binding mycotoxins, modulating gut microbiota, improving gut morphology and reducing inflammatory responses (Jensen and Keasling, 2015). Considerable variation exists among yeast strains and products in their aflatoxin binding capacity (Shetty and Jespersen, 2006). Yeast products from different industrial sources reduced AFM₁ concentration in milk by 45-70%, indicating product-specific efficacy (Gonçalves *et al.*, 2017). The effects of dietary inclusion of *Saccharomyces cerevisiae* on dry matter intake, aflatoxin intake, milk yield, aflatoxin excretion in milk, carry-over rate from feed to milk, and the milk somatic cell count (SCC) observed under the current study are presented in Table 1.

Dry Matter and Aflatoxin Intake

Dry matter intake was significantly (p<0.05) lower in the aflatoxin-fed control group (T₁). Supplementation with *Saccharomyces cerevisiae* significantly improved DMI, with the highest intake recorded in T₃ (0.1%). Reduced feed intake in aflatoxin-fed animals may be attributed to systemic stress



and endocrine disturbances affecting metabolism (Eraslan *et al.*, 2006). Similar reductions have been reported in cattle and buffaloes fed aflatoxin-contaminated diets (Pasha, 2008; Pastorelli *et al.*, 2012; Akhtar *et al.*, 2014).

Aflatoxin intake differed significantly ($p < 0.05$) among treatments and followed the same trend as DMI. The highest intake was observed in T₃ due to greater feed consumption, indicating that yeast supplementation did not reduce aflatoxin intake per se but mitigated its biological effects.

Milk Yield, Aflatoxin M₁ Excretion in Milk and Carry-Over Rate

Milk yield was significantly ($p < 0.05$) lower in the aflatoxin-fed control group. Inclusion of *Saccharomyces cerevisiae* significantly improved milk yield, with T₃ showing the highest yield. Aflatoxin-induced reduction in milk production has been widely reported (Queiroz *et al.*, 2012; Malinee *et al.*, 2020). Improved milk yield in yeast-supplemented groups may be attributed to enhanced nutrient utilisation and reduced toxin bioavailability.

AFM₁ excretion was significantly ($p < 0.05$) higher in the control group. Inclusion of *Saccharomyces cerevisiae* at 0.1 and 0.2% significantly reduced AFM₁ excretion, indicating effective binding of AFB₁ in the gastrointestinal tract and reduced transfer into milk.

The carry-over rate of aflatoxin from feed to milk was highest in T₁ and significantly reduced in yeast-supplemented groups, with the lowest value recorded in T₃. These results were consistent with earlier reports (Bibyan *et al.*, 2023) and confirm the efficacy of *Saccharomyces cerevisiae* in reducing aflatoxin transmission.

Somatic Cell Count

Somatic cell count was significantly ($p < 0.05$) higher in the control group, indicating compromised mammary health.

Inclusion of *Saccharomyces cerevisiae* at 0.1 and 0.2% significantly reduced SCC, consistent with earlier findings linking aflatoxin exposure to increased SCC (Lafont *et al.*, 1983).

Haematological Parameters

Aflatoxin contamination significantly reduced RBC, WBC, haemoglobin and haematocrit values of lactating cows, indicating anaemia and suppression. Yeast supplementation at all levels effectively ameliorated these effects (Table 2), consistent with earlier reports (Abdel-Rahman and Okle, 2014; Naseer *et al.*, 2018).

Biochemical Parameters

Serum total protein, glucose and cholesterol levels of cows were found to be lower in aflatoxin contaminated feed fed group T₁, while in T₃ and/or T₄ groups, these were increased significantly. However, there was no influence of yeast supplement on the serum concentration of creatinine and BUN (Table 3). The lack of significant differences in serum creatinine and BUN concentrations among treatments suggests that yeast supplementation did not affect renal function or systemic nitrogen metabolism, as the primary action of *Saccharomyces cerevisiae* is mainly confined to modulation of rumen microbial colonization and fermentation rather than post-absorptive metabolic processes (Chaucheyras-Durand and Fonty, 2002)

Furthermore, the elevated serum enzyme alkaline phosphatase, aspartate and alanine aminotransferase (AST-ALT), including lactate dehydrogenase and acetylcholinesterase (AChE) activities in T₁ indicate hepatic damage due to aflatoxicosis (Silambarasan *et al.*, 2016; Singh, 2019). Yeast supplementation significantly reduced enzyme activities, with 0.2% inclusion being the most effective for hepatic enzymes (Table 4).

Table 1: Dry matter intake (DMI), aflatoxin intake (AFI), average milk yield (MY), aflatoxin excretion (AFE), carry-over % and milk SCC in cattle under different yeast treatment

Treatment	DMI (kg/d)	AFI (µg/d)	Average MY (L)	AFE (µg/d)	Carry-over (%)	SCC (×10 ⁵ cells/mL)
T ₁	10.08 ^a ±0.02	312.54 ^a ±0.70	4.14 ^a ±0.02	13.08 ^c ±0.17	0.84 ^c ±0.01	2.53 ^b ±0.01
T ₂	10.24 ^b ±0.01	317.50 ^b ±0.60	4.31 ^b ±0.01	11.17 ^b ±0.31	0.70 ^b ±0.02	2.50 ^b ±0.01
T ₃	10.39 ^c ±0.01	322.09 ^c ±0.38	4.42 ^c ±0.02	8.74 ^a ±0.13	0.54 ^a ±0.01	2.33 ^a ±0.02
T ₄	10.33 ^{bc} ±0.05	320.23 ^{bc} ±1.78	4.29 ^b ±0.01	9.18 ^a ±0.13	0.57 ^a ±0.01	2.35 ^a ±0.01

T₁=control diet, T₂, T₃, T₄=*Saccharomyces cerevisiae* @ 0.05, 0.1 & 0.2% in feed. Values bearing different superscripts within a column differ significantly ($p < 0.05$).

Table 2: Effect of inclusion of *Saccharomyces cerevisiae* on haematological parameters of lactating cattle

Treatment	RBC (×10 ⁶ /µL)	WBC (×10 ³ /µL)	Hb (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g%)
T ₁	5.29 ^a ±0.04	6.39 ^a ±0.13	7.67 ^a ±0.03	28.17 ^a ±0.02	53.21 ^b ±0.34	14.49 ^a ±0.12	27.44 ^a ±0.24
T ₂	5.63 ^b ±0.04	6.76 ^b ±0.08	8.23 ^b ±0.04	29.07 ^b ±0.18	51.63 ^a ±0.60	14.62 ^a ±0.12	28.32 ^b ±0.19
T ₃	5.71 ^b ±0.03	6.95 ^b ±0.02	8.35 ^b ±0.05	29.64 ^c ±0.12	51.87 ^a ±0.24	14.62 ^a ±0.11	28.19 ^{ab} ±0.17
T ₄	5.72 ^b ±0.03	6.97 ^b ±0.02	8.35 ^b ±0.07	29.62 ^c ±0.12	51.76 ^a ±0.34	14.59 ^a ±0.16	28.19 ^{ab} ±0.33

Values bearing different superscripts within a column differ significantly ($p < 0.05$).

Table 3: Effect of inclusion of *Saccharomyces cerevisiae* on total serum protein, glucose, cholesterol, creatinine and urea content in lactating cows

Treatment	Total Protein (g/dL)	Glucose (mg/dL)	Cholesterol (mg/dL)	Creatinine (µmol/L)	Urea (mmol/L)
T ₁	5.17 ^a ±0.01	62.67 ^a ±0.23	113.73 ^{ab} ±2.11	60.40 ^a ±0.50	3.73 ^a ±0.16
T ₂	5.25 ^a ±0.02	63.35 ^a ±0.47	109.77 ^a ±1.72	60.20 ^a ±0.73	3.55 ^a ±0.19
T ₃	5.50 ^b ±0.03	63.57 ^a ±0.46	116.92 ^c ±1.55	59.80 ^a ±1.06	3.47 ^a ±0.15
T ₄	5.51 ^b ±0.04	65.18 ^b ±0.34	112.10 ^{ab} ±0.85	60.00 ^a ±0.71	3.46 ^a ±0.16

Values bearing different superscripts within a column differ significantly (p < 0.05).

Table 4: Effect of addition of *Saccharomyces cerevisiae* in feed on serum alkaline phosphatase (ALP), aspartate and alanine aminotransferase (AST-ALT), lactate dehydrogenase (LDH) and acetylcholinesterase (AChE) activities of cows

Treatment	ALP (IU/L)	AST (IU/L)	ALT (IU/L)	LDH (IU/L)	AChE (IU/L)
T ₁	88.26 ^d ±0.38	107.36 ^d ±1.13	56.69 ^b ±1.3	163.56 ^b ±1.15	206.07 ^b ±1.85
T ₂	85.72 ^c ±0.41	101.92 ^c ±1.16	54.92 ^b ±0.74	155.82 ^a ±1.73	194.36 ^a ±1.49
T ₃	81.84 ^b ±0.39	96.61 ^b ±0.99	50.70 ^a ±0.40	154.96 ^a ±1.43	194.57 ^a ±1.53
T ₄	78.63 ^a ±0.80	91.82 ^a ±0.92	50.57 ^a ±1.34	159.21 ^a ±1.07	197.36 ^a ±1.98

Values bearing different superscripts within a column differ significantly (p < 0.05)

CONCLUSION

Dietary supplementation of *Saccharomyces cerevisiae* effectively mitigated the adverse effects of AFB₁ in lactating dairy cattle. Yeast inclusion significantly improved dry matter intake, enhanced milk yield, and reduced AFM₁ excretion and carry-over rates. Additionally, yeast supplementation ameliorated hepatic enzyme elevations, improved biochemical profiles, and normalised haematological parameters, indicating reduced aflatoxicosis-induced stress. The lowest somatic cell count in yeast-supplemented groups further highlights improved mammary health. The study confirms that *Saccharomyces cerevisiae* at 0.1-0.2% of the diet is an efficacious and practical strategy to enhance milk safety and dairy cow performance in aflatoxin-exposed feeding systems.

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